

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Lorin R. DeBonte, et al. Art Unit : Unknown
Serial No. : Examiner : Unknown
Filed :
Title : FATTY ACID DESATURASES AND MUTANT SEQUENCES THEREOF

Commissioner for Patents
Washington, D.C. 20231

PRELIMINARY AMENDMENT

In the Specification

After the specification and before the claims please insert the enclosed Sequence Listing as pages 51-81. Please renumber original pages 51-59 as 82-90, respectively.

At page 1, line 26, please delete "In." and insert therefor --In--.

At page 8, line 11, please delete "on" and insert therefor --as--.

At page 11, line 7, please delete "WO 94/115116" and insert therefore --WO 94/11516--.

At page 12, line 12, please delete "WO 94/115116" and insert therefore --WO 94/11516--.

At page 43, lines 16-19, please delete "non-polar residue, leucine, for a polar residue, histidine" and insert therefor --polar residue, histidine, for a non-polar residue, leucine--.

At page 44, line 23, after "3'", please insert --(SEQ ID NO:19--). At line 24, after "3'", please insert --(SEQ ID NO:20--). At line 26, after "3'", please insert --(SEQ ID NO:21)--. At line 27, after "3'", please insert --(SEQ ID NO:22)--.

At page 49, line 7, after "CAUCAUCAUCAUCCTTCTCGTAGGGTTCATCG", please insert --(SEQ ID NO:23)--. At line 8, after

"CUACUACUACUATCATAGAAGAGAAAGGTTCAG", please insert --(SEQ ID NO:24)--.

At line 13, after "3'", please insert --(SEQ ID NO:25)--. At line 14, after "3'", please insert --(SEQ ID NO:26)--.

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No new matter is added by these amendments.

In the Claims

Please cancel claims 10, 15, 20-25, 27, and 31 without prejudice to further prosecution.

Please amend the claims as follows:

1. (Amended) An isolated nucleic acid fragment comprising a sequence of at least about 10 nucleotides from a Brassicaceae or Helianthus delta-12 fatty acid desaturase gene having at least one mutation in a region of said desaturase gene encoding a His-Xaa-Xaa-Xaa-His amino acid motif, wherein said [gene is effective for altering fatty acid composition in Brassicaceae or Helianthus seeds] at least one mutation renders the product of said desaturase gene non-functional and wherein said sequence includes said at least one mutation.

3. (Amended) The nucleic acid fragment of claim 2, wherein said at least one mutation [comprises] in said gene introduces a non-conservative amino acid substitution in said [region] motif.

4. (Amended) An isolated nucleic acid fragment comprising a sequence of at least about 10 nucleotides from a Brassicaceae or Helianthus delta-15 fatty acid desaturase gene having at least one mutation in a region of said desaturase gene encoding a His-Xaa-Xaa-Xaa-His amino acid motif, wherein said [gene is effective for altering fatty acid composition in Brassicaceae or Helianthus seeds] at least one mutation renders the product of said desaturase gene non-functional and wherein said sequence includes said at least one mutation.

6. (Amended) The nucleic acid fragment of claim 5, wherein said at least one mutation [comprises] in said gene introduces a non-conservative amino acid substitution in said [region] motif.

7. (Amended) An isolated nucleic acid fragment encoding a polypeptide having an amino acid sequence selected from the group consisting of: [an amino acid sequence substantially identical to] SEQ ID NO:12, [an amino acid sequence substantially identical to] and SEQ ID NO:16 [and an amino acid sequence substantially identical to SEQ ID NO:18].

11. (Amended) An isolated nucleic acid fragment, wherein said nucleic acid fragment is selected from the group consisting of:

- a) SEQ ID NO:11;
- b) SEQ ID NO:15;
- c) [SEQ ID NO:17;
- d)] an RNA [analog] comprising a nucleotide sequence of SEQ ID NO:11,
wherein uracil replaces thymine;

[e]d) an RNA [analog] comprising a nucleotide sequence of SEQ ID NO:15,
wherein uracil replaces thymine;

- [f] an RNA analog of SEQ ID NO:17;
- g]e) a nucleic acid fragment having a nucleic acid sequence complementary to a),
b), c), or d)[, e), or f]; and

[h]f) a nucleic acid fragment of a), b), c), d), or e)[, f), or g,] that is at least 10 nucleotides in length and that hybridizes under stringent conditions to genomic DNA encoding the mutation in the polypeptide of SEQ ID NO:12, or SEQ ID NO:16[, or SEQ ID NO:18].

12. (Amended) An isolated polypeptide having an amino acid sequence selected from the group consisting of: [an amino acid sequence substantially identical to] SEQ ID NO:12[,] and [an amino acid sequence substantially identical to] SEQ ID NO:16[, and an amino acid sequence substantially identical to SEQ ID NO:18].

16. (Amended) A Brassicaceae or Helianthus plant, said plant containing first and second delta-12 fatty acid desaturase genes, each said gene having at least one mutation, wherein at least one of said mutations is in a region encoding a His-Xaa-Xaa-Xaa-His amino acid motif and wherein each said mutation renders the product of said desaturase gene non-functional, and confers an altered fatty acid composition in seeds of said plant.

In claim 18, please delete "region" and insert therefor --motif--

26. (Amended) A method for producing a Brassicaceae or Helianthus plant line, comprising the steps of:

- a) inducing mutagenesis in cells of a starting variety of a Brassicaceae or Helianthus species;
- b) obtaining one or more progeny plants from said cells;
- c) identifying at least one of said progeny plants that contain[s] a delta-12 fatty acid desaturase gene having at least one mutation, said at least one mutation in a region encoding a His-Xaa-Xaa-His amino acid motif, and wherein said mutation renders the product of said desaturase gene non-functional; and
- d) producing said plant line from said at least one progeny plant by self- or cross-pollination, said plant line having said at least one delta-12 gene mutation and producing seeds yielding an oil having a linoleic acid content from about 1% to about 14%.

28. (Amended) The method of claim 26, further comprising the steps of:

- e) inducing mutagenesis in cells of said plant line;
- f) obtaining one or more progeny plants from said plant line cells;
- g) identifying at least one of said plant line progeny plants that contains a delta-15 fatty acid desaturase gene having at least one delta-15 gene mutation, said at least one delta-15 gene mutation in a region encoding a His-Xaa-Xaa-His amino acid motif, wherein said mutation renders the product of said delta-15 desaturase gene non-functional;
- h) producing a second plant line from said at least one plant line progeny plant by self- or cross-pollination, said second plant line having said at least one delta-12 gene mutation and said at least one delta-15 gene mutation and producing seeds yielding an oil having a linoleic acid content from about 1% to about 14%.

30. (Amended) A method for producing a Brassicaceae plant line, comprising the steps of:

- a) inducing mutagenesis in cells of a starting variety of a Brassicaceae species;
- b) obtaining one or more progeny plants from said cells;

c) identifying at least one of said progeny plants that contains a delta-12 fatty acid desaturase gene having at least one mutation, said at least one mutation in a region encoding a His-Xaa-Xaa-Xaa-His amino acid motif, wherein said at least one mutation renders the product of said desaturase gene non-functional;

d) producing a first plant line from said at least one progeny plant by self- or cross-pollination, said plant line having said at least one delta-12 gene mutation;

e) inducing mutagenesis in cells of said first plant line;

f) obtaining one or more progeny plants from said first plant line cells;

g) identifying at least one of said first plant line progeny plants that contains a second delta-12 fatty acid desaturase gene having at least one mutation, said second gene mutation in a region other than a region encoding a His-Xaa-Xaa-Xaa-His amino acid motif; and

h) producing a second plant line from said at least one plant line progeny plant by self- or cross-pollination, said second plant line having said first delta-12 gene mutation and said second delta-12 gene mutation.

Please add the following new claims.

35. The nucleic acid fragment of claim 11, wherein said nucleic acid fragment is SEQ ID NO: 11.

36. The nucleic acid fragment of claim 11, wherein said nucleic acid fragment is SEQ ID NO: 15.

37. The plant of claim 16, wherein said plant is a Brassicaceae plant.

38. The plant of claim 37, wherein said plant is a Brassica napus plant.

39. The plant of claim 37, wherein said motif comprises the sequence His-Glu-Cys-Gly-His.

40. The plant of claim 37, wherein said mutation comprises a non-conservative amino acid substitution in said motif.

41. The method of claim 26, wherein said starting variety is a Brassicaceae species.

42. The method of claim 41, wherein said identifying step comprises identifying a mutation in a His-Glu-Cys-Gly-His amino acid motif.

43. The method of claim 28, wherein said starting variety is a Brassicaceae species variety.

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44. The method of claim 43, wherein said identifying step comprises identifying a mutation in a His-Glu-Cys-Gly-His amino acid motif.

45. The method of claim 43, wherein said producing step h) comprises producing seeds yielding an oil having an a-linolenic acid content from about 0.5% to about 10%.

46. The method of claim 30, wherein said identifying step b) comprises identifying a mutation in a His-Glu-Cys-Gly-His amino acid motif.

REMARKS

Applicants have amended claims 1, 3-4, 6-7, 16, 18, 26, 28, and 30, and cancelled claims 10, 15, 20-25, 27, and 31 without prejudice to further prosecution. Claims 35-46 are submitted herewith. Applicants respectfully request consideration and allowance of claims 1-9, 11-14, 16-19, 26, 28-30, and 32-46.

Claims 1 and 4 have been amended to recite that the mutation is in a region of the desaturase gene encoding a His-Xaa-Xaa-Xaa-His amino acid motif. Claims 1, 4, 16, 26, and 30 have been amended to recite that the mutation renders the desaturase gene non-functional. Support for these amendments can be found, for example, at page 8, lines 26-29, and at page 10, lines 29-33.

The amendments to the specification at pages 1, 8, 11-12, 43, 44 and 49 serve to correct typographical errors and add sequence identifiers.

Please apply any charges or credits to Deposit Account No. 06-1050.

Respectfully submitted,

Date: Toronto, 29, 2001

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